Effect of dual tocolysis with fenoterol and atosiban in human myometrium

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Abstract: Objectives To measure the tocolytic effect of the combination of the oxytocin receptor antagonist atosiban with the -mimetic agent fenoterol on human myometrium of pregnant women. Methods An in vitro study of contractility in human myometrium at the Laboratory of the Department of Obstetrics, University Hospital of Zürich, Switzerland, was performed. Thirty-six human myometrial biopsies were obtained during elective caesarean sections of singleton pregnancies at term. Tissue samples were exposed to atosiban, fenoterol and the combination of atosiban with fenoterol. Contractility was measured as area under the curve during 30 min of spontaneous contractions. The effect of treatment was expressed as the percentage of change from basal activity during 30 min of exposure. Differences were calculated using a paired Wilcoxon signed-rank test. An additive effect of dual tocolysis was assumed when no significant difference was detected between the observed and expected inhibition of dual tocolysis. When inhibition was greater or lower than expected, the dual combination was characterised as "synergistic" or "antagonistic", respectively. Results Atosiban and fenoterol alone suppressed contractions by a median of 43.2% and 29.8%, respectively. The combination of atosiban plus fenoterol was measured at a level of 67.3% inhibition. There was no significant difference in the expected (63.2%) and observed inhibition effect of dual tocolysis (P=0.945). Conclusions This study demonstrated an additive effect of dual tocolysis of atosiban and fenoterol on human myometrium in vitro, but no synergistic or antagonistic effect.

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Abstract

Objectives: To measure the tocolytic effect of the combination of the oxytocin receptor antagonist atosiban with the β-mimetic agent fenoterol on human myometrium of pregnant women.

Methods: An *in vitro* study of contractility in human myometrium at the Laboratory of the Department of Obstetrics, University Hospital of Zürich, Switzerland, was performed. Thirty-six human myometrial biopsies were obtained during elective caesarean sections of singleton pregnancies at term. Tissue samples were exposed to atosiban, fenoterol and the combination of atosiban with fenoterol. Contractility was measured as area under the curve during 30 min of spontaneous contractions. The effect of treatment was expressed as the percentage of change from basal activity during 30 min of exposure. Differences were calculated using a paired Wilcoxon signed-rank test. An additive effect of dual tocolysis was assumed when no significant difference was detected between the observed and expected inhibition of dual tocolysis. When inhibition was greater or lower than expected, the dual combination was characterised as "synergistic" or "antagonistic", respectively.

Results: Atosiban and fenoterol alone suppressed contractions by a median of 43.2% and 29.8%, respectively. The combination of atosiban plus fenoterol was measured at a level of 67.3% inhibition. There was no significant difference in the expected (63.2%) and observed inhibition effect of dual tocolysis (P = 0.945).

Conclusions: This study demonstrated an additive effect of dual tocolysis of atosiban and fenoterol on human myometrium *in vitro*, but no synergistic or antagonistic effect.

Keywords: *in vitro*; preterm delivery; tocolysis.

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Introduction

Preterm birth remains a problem for obstetric care. It accounts for 11.1% of births globally, and affects an estimated 14.9 million babies every year [1]. Preterm birth represents the single largest cause of mortality and morbidity in newborn babies and is a major cause of morbidity for pregnant women [2]. Data from developed countries showed an increase in the incidence of preterm births during the last decade. A population-based study in Denmark showed that the overall proportion of preterm deliveries increased by 22% from 1995 to 2004 [3].

Tocolytic agents play an essential role in hindering preterm deliveries. They have been shown to effectively delay delivery for at least 48 h, which enables administration of antenatal corticosteroids to enhance fetal lung maturation, as well as intrauterine transfer of the fetus to a tertiary care centre [4]. There is evidence that prolonging the pregnancy can decrease preterm delivery that is associated with severe infant morbidity and mortality, particularly during early gestation. Unfortunately, single tocolytic agents are not always effective at achieving this goal. Furthermore, some of these agents have substantial side effects that necessitate treatment cessation [5]. As an alternative, investigations into the use of combining two tocolytic agents have been gaining interest. The aims are twofold: to find ways to augment the tocolytic effect in patients with persisting contractions despite the maximum dose of a single tocolytic agent, and to reduce side effects using a lower dose of each single agent in combined therapies.

The main tocolytic agents currently in use alter myometrial contractility through two different pathways. First, they affect the contractile proteins (usually the phosphorylation of myosin) in a similar way to β-mimetic agents, by intracellular accumulation of cyclic adenosine

monophosphate (cAMP). The second pathway is to act on intracellular calcium concentrations, which can be influenced directly by calcium channel blockers or indirectly by preventing an increase in intracellular calcium via the inositol triphosphate production (IP3)-pathway, just as atosiban acts as an oxytocin receptor antagonist [5]. Simultaneous blockage of these two different pathways has been hypothesised as a promising combination for achieving an additive or even synergistic effect, with considerable benefit for clinically high risk situations.

In animal models, the combination of the β-mimetic agent ritodrine with atosiban resulted in synergistic inhibition of myometrial contractility, exceeding the additive effect [6]. While some physicians use this off-protocol combination therapy in complex situations in clinical practice, there is no experimental or clinical data about the effects in humans. The aim of this study was to analyse the *in vitro* tocolytic effects of the combination of atosiban with fenoterol in human myometrium of pregnant women. We hypothesised that due to different pathways of action an additive or even synergistic tocolytic effect could result.

Materials and methods

This *in vitro* study of contractility in human myometrium was performed at the Laboratory of the Department of Obstetrics, University Hospital of Zürich, Switzerland between 2012 and 2016. Myometrial biopsies were obtained from 36 women at a mean age of 33 years (SD 5.5) during elective caesarean section at term. All patients included in the study gave written informed consent. Excluded from the analysis were women who had tocolytic treatment within 2 weeks before caesarean section, previous caesarean section, preterm rupture of membranes, chorioamnionitis, preeclampsia or an infectious maternal disease.

The applied *in vitro* model for evaluation of tocolytic agents has been previously described in detail [7, 8]. In summary, biopsies of myometrial tissue (5 g) were taken from the superior edge of the hysterotomy incision, immediately immersed in iced Ringer or modified Krebs solution (NaCl 118; KCl 4.7; $CaCl_2$ 2.48; KH_2PO_4 1.24; $MgSO_4$ 1.21; $NaHCO_3$ 24.9; glucose 10.0; EDTA 0.034 mmol/L) and then transported within 2 h to the laboratory for further processing. Longitudinal strips of muscle fibres (approx. 15 × 2 × 1 mm) were carefully prepared. Four strips were simultaneously mounted into different chambers of a myograph (DMT800MS Muscle Strip Myograph system, DMT, ADInstruments GmbH, Aarhus, Denmark) and placed in a jacketed organ bath with gas feed lines. A heating thermostat maintained the Krebs solution at a temperature of 37°C and continuous aeration with oxycarbon (5% CO_2 and 95“/” 02, Pan Gas, Dagmarsellen, Switzerland) maintained a pH level of approximately 7.40. Tension was applied to the strips to initiate spontaneous contractions. Isometric contractions were recorded by the myograph and transferred digitally by a transducer (power rennet 4/30).

After measuring basal contractility for 30 min (basal activity – spontaneous contractions), the experimental substance was added and contractions were recorded again for another 30 min. Strips were exposed to the following substances: (1) atosiban (450 ng/mL, Ferring Pharmaceuticals A/S, Copenhagen, Denmark), (2) fenoterol (2242 pg/mL, Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim am Rhein, Germany), (3) a combination of atosiban and fenoterol, and (4) Krebs-Henseleit solution as an internal control. The concentration levels were determined based on the standard of care for clinical presentation of persisting contractions, despite administration of the maximum dose of tocolytic treatment [9, 10]. To test the viability of the samples, there was a 30-min wash out period during which the strips recovered and were able to resume regular spontaneous contractions. If myometrial strips did not continually contract until this last step of the experiment, they were excluded from further analysis.

Recorded myograms were analysed using LabChart Pro (ADInstruments, Dunedin, New Zealand) software. Contractility was defined as “area under the curve” of contractions. The tocolytic effect was defined as percent inhibition of contractility. The expected effect of dual-agent tocolysis was calculated as the sum of inhibition produced by each drug alone. The expected additive tocolytic effect was compared to the observed effect and the following three scenarios were possible: (1) additive effect – no significant difference between expected and observe effect; (2) synergistic effect – observed effect significantly superior to expected effect; or (3) inhibitory effect – observed effect significantly inferior to expected effect.

The observed inhibition was compared to the expected inhibition using the paired Wilcoxon signed-rank test. Statistical analysis was conducted using Sigmaplot 12.0 (Systat Software Inc., San Jose, CA, USA). The level of statistical significance was set at $P < 0.05$.

The study was approved by the local Ethics Committee (Kantonale Ethikkommission Zürich, No. 2012-0258) on 7th of August, 2012.

Results

The mean gestational age was 38 weeks (SD 0.8). The indications for caesarean section were breech position [11], psychosocial [11], pelvic disproportion [3], traumatic obstetric history [2], fetal malformation [1], maternal paraplegia [1] and fetal transverse presentation [1].

A total of 36 experiments were performed, five of which were done to establish the protocol and to validate the concentrations of test substances. From the remaining 31 biopsied tissue samples, eight had to be excluded because of insufficient or inconsistent contractions. As a result, 23 samples were available for analysis ($n = 23$).

Atosiban and fenoterol alone suppressed spontaneous contractions by a median of 43.2% and 29.8%, respectively. The combination of atosiban plus fenoterol reached a median level of 67.3% inhibition. The expected effect of dual-agent tocolysis (63.2%) was not significantly different from the observed effect ($P = 0.945$). Thus, an additive but not synergistic effect of dual tocolysis was found (Table 1, Figure 1).

Table 1: Percent inhibition of spontaneous contractions.

	Median	25%Q	75%Q
Atosiban alone	43.2	3.2	60.7
Fenoterol alone	29.8	9.6	73.0
Calculated additive tocolytic effect	63.2	13.6	95.9
Measured additive tocolytic effect	67.3	23.3	93.5
Control	−11.4	−40.1	21.2

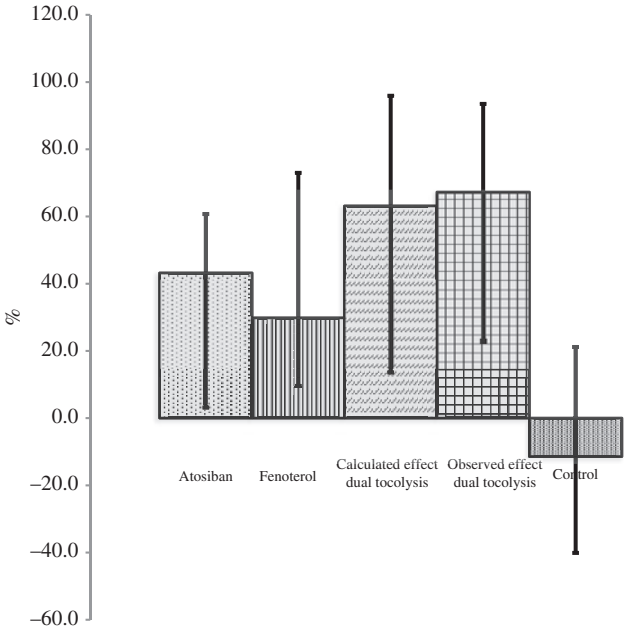

Figure 1: Inhibition of spontaneous contractions after addition of atosiban or fenoterol alone, atosiban and fenoterol in combination and calculated additive reduction.
Data are expressed as median and interquartile range.

Likewise, the frequency of contractions was suppressed by tocolytic agents at a similar scale. The expected effect of dual-agent tocolysis was not significantly different from the observed effect (P = 0.71, data not shown).

Discussion

This is the first study to analyse the tocolytic effect of the combination of atosiban with fenoterol on human myometrium. While we demonstrated that dual tocolysis of atosiban plus fenoterol can achieve an additive effect to suppress contractions in spontaneously contracting human myometrium, a synergistic effect was not found.

The *in vitro* model is well established at our institute and internationally accepted in the literature as a means to evaluate the effect of single or dual tocolysis [6, 7, 11–14]. However, this *in vitro* model can only approximate the clinical situation in which preterm birth is a pathophysiologically heterogenous syndrome [15].

The rationale for a possible benefit of combining two tocolytic agents is based on two main assumptions: (1) the combination of agents leads to an additive or even synergistic effect with a superior clinical outcome in cases when a single agent is not effective, and (2) the combination of tocolytic agents may permit a dose reduction of both agents while having the same tocolytic effect, which would lead to a decrease in maternal and fetal side effects. This may also decrease the costs associated with tocolysis (high costs are a limiting factor for the use of atosiban). Tocolytics act through different pathways to inhibit uterine contractile activity. β-Mimetics induce relaxation through intracellular accumulation of cAMP [16]. Calcium channel blockers directly prevent influx of calcium ions responsible for smooth muscle cell contraction, whereas oxytocin receptor antagonists act indirectly to prevent the rise of intracellular calcium concentration in response to the oxytocin-induced increase in lP3 [17]. Simultaneous blockage of different pathways could result in a synergistic effect capable of potentiating the uterine relaxation induced by each single drug.

Despite the possible advantages, only a few clinical studies have evaluated the effects of a combination of tocolytics. Evidence for the effect of combined tocolytics has been reviewed recently in a Cochrane analysis [18]. No difference in infant outcomes was reported for any of the test combinations. Only the combination of ritodrine plus vaginal progesterone showed a positive effect. The authors noted that there were no trials using a combination regimen with widely used tocolytic agents, such as calcium channel blockers or oxytocin receptor antagonists. Recently, a combination of salbutamol and nifedipine was tested in a small prospective cohort study [19]. The authors found no significant clinical difference when salbutamol was added before additional adverse effects were experienced.

While the paucity of clinical studies evaluating a combination of tocolytic agents may be due to ethical considerations about enrolling pregnant women at high risk for preterm birth in randomised controlled trials, *in vitro* models allow for testing of substances on myometrium to identify the most promising combinations before *in vivo* testing. To date, seven *in vitro* studies have been published evaluating the effects of combining tocolytics on isolated myometrium. The combination of β-mimetics with nifedipine demonstrated inhibition of contraction superior to either agent alone [20, 21]. Doret et al. were the first to classify the tocolytic effect of a combination to be

antagonistic, additive or synergistic, as described above. The authors could demonstrate that in rat myometrium the combinations of either rofecoxib or atosiban with ritodrine had a synergistic effect while other combinations resulted in an additive effect [6, 22]. Two more recent *in vitro* studies on human myometrium demonstrated that atosiban and nifedipine resulted in an additive tocolytic effect and nifedipine with ritodrine produced a significantly greater inhibition of contractility than each drug alone [11, 14]. In conclusion, there were two promising combinations that exhibited a synergistic inhibition for myometrial activity: ritodrine with rofecoxib and ritodrine with atosiban. Rofecoxib is not used in clinical practice as a tocolytic agent as severe adverse effects have been described [23].

Therefore, the combination of a β-mimetic and atosiban seemed to be the most promising at achieving maximal tocolytic effects in clinically difficult situations when a single agent was not sufficient to supress contractions, or when side effects or monetary restrictions prevent maximal dosage of single agents. Considering the promising results in the animal model, we conducted our analysis to project the possible *in vivo* reaction [9, 10].

In our study we tried to approximate a clinical situation by using human myometrium and drug concentrations measured *in vivo*. However, there are no available data about pharmacocinetic interactions between atosiban and fenoterol. One study analysed the interaction between atosiban and the β-blocking agent lalol. While Cmax was reduced and tmax was prolonged for labetalol, the authors concluded that bioavailability was not influenced in a clinically relevant matter [24]. Even though the β-mimetic fenoterol is not comparable to the β-blocking labetalol, we cannot exclude an interaction *in vivo* and our *in vitro* model is not sufficient for a final assessment of the effectiveness of dual tocolysis. Furthermore, it is known that receptor mediated contractility of human myometrium changes in the course of pregnancy [25]. Myometrial biopsies used for our experiments were taken at term during elective caesarean sections to guarantee the ethical standard of an informed consent that was requested the day before surgery. Our results should be interpreted considering the possible difference in contractility and receptor expression in preterm myometrium.

Test substances were applied on spontaneously contracting human myometrial samples. This was contrary to methods of other investigators using a model of oxytocin-induced contractions to analyse tocolytic effects [12–14]. In preliminary experiments, however, we found that this approach was not feasible for a study design including atosiban as oxytocin-induced contractions were already maximally supressed by the oxytocin receptor antagonist atosiban (data not shown). In our *in vitro* human model, however, a synergistic effect, as described in rat myometrium, could not be confirmed.

Clinical studies are needed to examine the *in vivo* effects, possible pharmacocinetic interactions and safety of the most promising combinations of tocolytics in the *in vitro* model, such as β-mimetic/atosiban or nifedipine/atosiban. Further *in vitro* experiments could also evaluate a combination of three substances including progesterone.

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References

1. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379:2162–72.
2. Lawn JE, Kerber K, Enweronu-Laryea C, Cousens S. 3.6 Million neonatal deaths – what is progressing and what is not? Semin Perinatol. 2010;34:371–86.
3. Langhoff-Roos J, Kesmodel U, Jacobsson B, Rasmussen S, Vogel I. Spontaneous preterm delivery in primiparous women at low risk in Denmark: population based study. Br Med J. 2006;332:937–9.
4. Gyetvai K, Hannah ME, Hodnett ED, Ohlsson A. Tocolytics for preterm labor: a systematic review. Obstet Gynecol. 1999;94:869–77.
5. Caritis S. Adverse effects of tocolytic therapy. BJOG 2005;112(Suppl 1):74–8.
6. Doret M, Mellier G, Gaucherand P, Saade GR, Benchaib M, Frutoso J, et al. The *in vitro* effect of dual combinations of ritodrine, nicardipine and atosiban on contractility of pregnant rat myometrium. BJOG 2003;110:731–4.

7. Wachter R, Brenneisen R, Hamburger M, Mennet M, Schnelle M, Worel AM, et al. Leaf press juice from *Bryophyllum pinnatum* (Lamarck) Oken induces myometrial relaxation. Phytomedicine. 2011;19:74–82.
8. Gwehenberger B, Rist L, Huch R, von Mandach U. Effect of *Bryophyllum pinnatum* versus fenoterol on uterine contractility. Eur J Obstet Gynecol Reprod Biol. 2004;113:164–71.
9. Goodwin TM, Valenzuela GJ, Silver H, Creasy G. Dose ranging study of the oxytocin antagonist atosiban in the treatment of preterm labor. Atosiban Study Group. Obstet Gynecol. 1996;88:331–6.
10. von Mandach U, Boni R, Danko J, Huch R, Huch A. Pharmacokinetics of fenoterol in pregnant women. Arzneimittelforschung. 1995;45:186–9.
11. Kuc P, Laudanski P, Pierzynski P, Laudanski T. The effect of combined tocolysis on in vitro uterine contractility in preterm labour. Adv Med Sci. 2011;56:88–94.
12. Arrowsmith S, Neilson J, Wray S. The combination tocolytic effect of MgSO4 and an oxytocin receptor antagonist in myometrium from singleton and twin pregnancies. Am J Obstet Gynecol. 2016;215:e1–9.
13. Buscher U, Chen FC, Riesenkampff E, von Dehn D, David M, Dudenhausen JW. Effects of oxytocin receptor antagonist atosiban on pregnant myometrium in vitro. Obstet Gynecol. 2001;98:117–21.
14. Carvajal JA, Zambrano MJ, Theodor NM, Moreno LE, Olguin TR, Vanhauwaert PS, et al. The synergic *in vitro* tocolytic effect of nifedipine plus ritodrine on human myometrial contractility. Reprod Sci. 2017;24:635–40.
15. Romero R, Mazor M, Munoz H, Gomez R, Galasso M, Sherer DM. The preterm labor syndrome. Ann N Y Acad Sci. 1994;734: 414–29.
16. Anotayanonth S, Subhedar NV, Garner P, Neilson JP, Harigopal S. Betamimetics for inhibiting preterm labour. Cochrane Database Syst Rev. 2004;4:CD004352.
17. Goodwin TM, Valenzuela G, Silver H, Hayashi R, Creasy GW, Lane R. Treatment of preterm labor with the oxytocin antagonist atosiban. Am J Perinatol. 1996;13:143–6.
18. Vogel JP, Nardin JM, Dowswell T, West HM, Oladapo OT. Combination of tocolytic agents for inhibiting preterm labour. Cochrane Database Syst Rev. 2014;7:Cd006169.
19. Ng QJ, Abdul Rahman MF, Lim ML, Chee JJ, Tan KH. Single versus combination tocolytic regimen in the prevention of preterm births in women: a prospective cohort study. J Perinat Med. 2015;43:423–8.
20. Gallagher LA, Gautieri RF. Influence of a combination of ritodrine and nifedipine on the isolated rat uterus. Res Commun Chem Pathol Pharmacol. 1992;75:3–18.
21. Saade GR, Taskin O, Belfort MA, Erturan B, Moise KJ Jr. In vitro comparison of four tocolytic agents, alone and in combination. Obstet Gynecol. 1994;84:374–8.
22. Doret M, Mellier G, Benchaib M, Piacenza JM, Gharib C, Pasquier JC. In vitro study of tocolytic effect of rofecoxib, a specific cyclo-oxygenase 2 inhibitor. Comparison and combination with other tocolytic agents. BJOG 2002;109:983–8.
23. Juni P, Nartey L, Reichenbach S, Sterchi R, Dieppe PA, Egger M. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. Lancet. 2004;364:2021–9.
24. Rasmussen BB, Larsen LS, Senderovitz T. Pharmacokinetic interaction studies of atosiban with labetalol or betamethasone in healthy female volunteers. BJOG 2005;112:1492–9.
25. Yulia A, Johnson MR. Myometrial oxytocin receptor expression and intracellular pathways. Minerva Ginecol. 2014;66: 267–80.